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REVIEW ARTICLE

Mammalian epoxide hydrolases in xenobiotic metabolism and signalling

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Abstract Epoxide hydrolases catalyse the hydrolysis of electrophilic—and therefore potentially genotoxic—epoxides to the corresponding less reactive vicinal diols, which explains the classification of epoxide hydrolases as typical detoxifying enzymes. The best example is mammalian microsomal epoxide hydrolase (mEH)—an enzyme prone to detoxification—due to a high expression level in the liver, a broad substrate selectivity, as well as inducibility by foreign compounds. The mEH is capable of inactivating a large number of structurally different, highly reactive epoxides and hence is an important part of the enzymatic defence of our organism against adverse effects of foreign compounds. Furthermore, evidence is accumulating that mammalian epoxide hydrolases play physiological roles other than detoxification, particularly through involvement in signalling processes. This certainly holds true for soluble epoxide hydrolase (sEH) whose main function seems to be the turnover of lipid derived epoxides, which are signalling lipids with diverse functions in regulatory processes, such as control of blood pressure, inflammatory processes, cell proliferation and nociception. In recent years, the sEH has attracted attention as a promising target for pharmacological inhibition to treat hypertension and possibly other diseases. Recently, new hitherto uncharacterised epoxide hydrolases could be identified in mammals by genome analysis. The expression pattern and substrate selectivity of these new epoxide hydrolases suggests their participation in signalling processes rather than a role in detoxification. Taken together, epoxide hydrolases (1) play a central role in the detoxification of genotoxic epoxides and (2) have an important function in the regulation of physiological processes by the control of signalling molecules with an epoxide structure.

Keywords Epoxide hydrolase · Xenobiotic metabolism · EPHX · ABHD · Lipid signalling · peg1/MEST · EET · Cholesterol · Lipid phosphatase

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Introduction

Epoxide hydrolases (EC 3.3.2.7-11) catalyse the hydrolysis of epoxides to the corresponding vicinal diols by the addition of water. Xenobiotic derived epoxides can be formed within the body as reactive intermediates during metabolic processes by monooxygenation of carbon double bonds in olefines or aromatic ring systems. Based on the ring tension of such cyclic compounds in combination with a polarisation of the CO bond, epoxides often show electrophilic reactivity leading to a certain genotoxic potential (Fig. 1a). Two mammalian enzymes—microsomal (mEH) and soluble epoxide hydrolase (sEH)—that classically play a major role in xenobiotic metabolism have been intensely characterised. Apart from a few exceptions the hydrolysis of an epoxide results in an increased water solubility of the metabolites and the termination of its genotoxic potential. Therefore mammalian epoxide hydrolases, in particular the mEH with its exceptionally broad substrate selectivity against a diverse group of epoxides, perfectly serve their function as xenobiotic metabolising enzymes (Fig. 1b).

This picture, however, needs some adjustment. It is now well established that the organism utilises a large number of endogenous epoxides—in particular epoxides derived from unsaturated fatty acids, such as epoxyeicosatrienoic acid

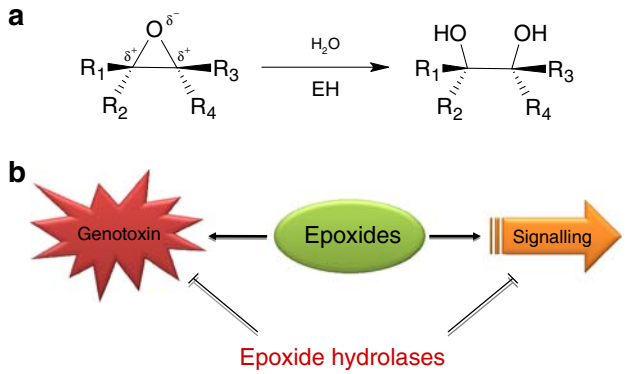

Fig. 1 The role of epoxide hydrolase in xenobiotic metabolism and cell signalling. **a** Epoxides are in general chemically reactive compounds, due to the electronegativity of the ring oxygen in combination with a certain ring tension. **b** Reactive epoxides may be taken up by the body as xenobiotic substrates or formed during metabolism, mainly by the action of cytochrome P450 dependant monooxygenases. On the other hand, many epoxides without any genotoxic potential have been identified as endogenous signalling molecules, which display numerous functions within the organism. The turnover of epoxide to the corresponding diols by epoxide hydrolases is most often considered a detoxifying reaction. Rather rare exceptions where (1) the action of an EH leads to metabolic bioactivation, or (2) the diol reaction product still has signalling function are outlined in the respective chapters in the text

(EETs) and leukotriene A_4 (LTA_4)—as signalling molecules. Due to their low chemical reactivity, these epoxides have little genotoxic potential, but instead serve as important signalling molecules, regulating a large variety of physiological functions, ranging from the regulation of vascular tone, to inflammation, angiogenesis and pain. Human sEH, which is highly expressed throughout the body, is to date regarded as the primary enzyme in the metabolism of such endogenous epoxides (Fig. 1b). Our recently discovered phosphatase activity of human sEH further highlights the role of this enzyme in regulatory processes rather than xenobiotic metabolism. Yet, it also serves a complementary function to mEH in xenobiotic metabolism due to the acceptance of certain *trans*-substituted epoxides.

Apart from the two well analysed EHs additional mammalian epoxide hydrolases contribute to the metabolism of epoxides. Both sEH and mEH show an overall low sequence homology but both belong to the family of α/β hydrolase fold enzymes, based on structural similarities. Using common structural elements of the α/β hydrolase fold, candidate enzymes from the mammalian genomes may be identified representing potential epoxide hydrolases. Our group has recently cloned and expressed two such genes that we name epoxide hydrolase 3 (EH3) and 4 (EH4) (manuscript in preparation) from the human genome. Furthermore, the product of the peg1/MEST gene was already in 1995 predicted to represent an α/β hydrolase fold epoxide hydrolase (Kaneko-Ishino et al. 1995). The physiological functions of these novel (potential) epoxide hydrolases in xenobiotic metabolism and/or lipid signalling still needs to be determined.

Additional epoxide hydrolases with narrow substrate selectivity—and therefore an unlikely role in xenobiotic metabolism—have been characterised in mammals, based on their enzymatic functions. Of those, mammalian leukotriene A_4 hydrolase (LTA_4H), which is involved in the turnover of the lipid mediator LTA_4 to LTB_4, is best characterised (Haeggstrom 2004). LTA_4 hydrolase does not belong to the α/β hydrolase fold enzyme family but instead represents a zinc dependant metalloprotease, which—as an exception compared to all other EHs—forms a non-vicinal diol from its substrate. Less well-investigated are Hepoxilin A_3 epoxide hydrolase and cholesterol epoxide hydrolase (ChEH) which were identified as the main hydrolase of the endogenous lipids hepoxilin A_3 (Pace-Asciak and Lee 1989) and cholesterol-5,6-epoxide (Watabe et al. 1986), respectively. To date, both enzymes are still only incompletely characterised and no sequence or structural information is available.

Most known epoxide hydrolases belong to the large family of α/β hydrolase fold enzymes and are found in nearly every organism (van Loo et al. 2006). Yet, in bacteria also different strategies for epoxide hydrolysis evolved. Such enzymes are generally specialised on a function in one specific pathway rather than accepting a broad spectrum of substrates. The limonene epoxide hydrolase (LEH) from *Rhodococcus erythropolis* (van der Werf et al. 1998), as well as a homologous EH (TbEH1) from *Mycobacterium tuberculosis* represent enzymes smaller than the general α/β hydrolase fold EHs, and utilise a single step mechanism for epoxide hydrolysis. A distinct class of EHs is represented by the FosX epoxide hydrolases from microorganisms such as *Mesorhizobium loti* and *Listeria monocytogenes* which selectively hydrolyses the antibiotic Fosfomycin by a mechanism including the direct addition of water (Fillgrove et al. 2003; Rigsby et al. 2005).

This review focuses on an update of the α/β hydrolase fold family of mammalian epoxide hydrolases and their role in xenobiotic metabolism, but will also summarise the functions of these enzymes which derive from hydrolysis of endogenous lipid mediators.

Nomenclature of epoxide hydrolases

Several epoxide hydrolases and their corresponding genes have been characterised in mammals. Historically, these enzymes were named after the species of origin, the subcellular localisation and/or their substrate specificity. Given that the present gene nomenclature is not consistent but most of the mammalian EHs belong to the α/β hydrolase

fold family of enzymes, a more systematic nomenclature based on evolutionary relation similar to the cytochrome P450 monooxygenases (CYP) nomenclature seems appropriate. The two well investigated mammalian EHs have been named EPHX1 (mEH) and EPHX2 (sEH) by the HUGO gene nomenclature committee, therefore we suggest that this nomenclature should also be used for new members of the α/β hydrolase fold EH enzyme family (Table 1). As mentioned earlier, we identified two new epoxide hydrolase genes (presently termed ABHD7 and ABHD9 based on a predicted α/β hydrolase fold) with 45% sequence identity in the mammalian genome (Fig. 2), at least one of which acts as an epoxide hydrolase on lipid derived epoxides (manuscript in preparation). Two structurally very closely related EHs from *Caenorhabditis elegans* have very recently been described (Harris et al. 2008) as active epoxide hydrolases. Therefore we suggest terming the two new mammalian enzymes EH3 and EH4 and propose changing their gene names into EPHX3 and EPHX4. Because for the peg1/MEST gene product the evidence for epoxide hydrolase activity has not been proven so far, and for ChEH and Hepoxilin A_3 hydrolase no sequence information is available yet, we propose terming the next sequence- and substrate-characterised α/β hydrolase fold epoxide hydrolase EH5.

Gene evolution and structure of α/β hydrolase fold epoxide hydrolases

The α/β hydrolase fold family comprises of a broad variety of enzymes with catalytic activities ranging from esterases (acetylcholinesterase being the most prominent) to epoxide hydrolases, haloalkane dehalogenases and lipases (Holmquist 2000). The sequence similarity of the first cloned epoxide hydrolase—mEH (Gonzalez and Kasper 1981)—and the first described bacterial haloalkane dehalogenase (Janssen et al. 1989) is minimal but a classification into the α/β hydrolase fold family of enzymes based on structural similarities (Arand et al. 1994; Lacourciere and Armstrong 1994; Beetham et al. 1995) was suggested after (1) cloning of the related sEH (Beetham et al. 1993; Grant et al. 1993; Knehr et al. 1993), and (2) determination of the crystal structure of haloalkane dehalogenase (Franken et al. 1991). The structural relationship of epoxide hydrolases has been described in detail in a recent review (Arand et al. 2003a). Since then approximately 240 epoxide hydrolase genes have been described from plants, fungi, insects and bacteria (Beetham et al. 1993, 1995; Smit 2004; van Loo et al. 2006). Another recent excellent review describes the genome wide analysis for epoxide hydrolases and evolutionary relationship of the identified enzymes with an average amino acid sequence identity of 14% into eight phylogenetically related groups (van Loo et al. 2006).

The first structure of an EH from *Agrobacterium radiobacter* (Nardini et al. 1999) with sequence similarity to mammalian sEH revealed typical structural elements of α/β hydrolase fold EHs. Common to all is a conserved fold consisting of an arrangement of eight central β-strands, flanked by several α-helices. A variable lid domain that is inserted between strand 6 and 7 covers this α/β hydrolase core fold (Holmquist 2000). Both, core and lid domains constitute the epoxide hydrolase substrate binding pocket at the interaction site. Similar in all family members is a catalytic triad formed by the central fold consisting of an Asp-His-Asp/Glu motif (catalytic nucleophile—water activating histidine—acidic residue, forming a charge relay system with

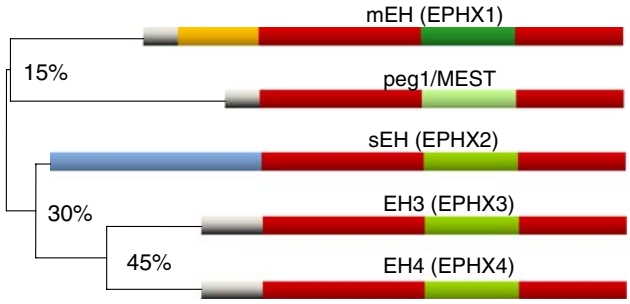

Fig. 2 Phylogenetic tree of mammalian epoxide hydrolases. Protein sequence comparison of human epoxide hydrolases sEH, mEH, EH3, EH4 as well as MEST in their α/β hydrolase fold domains (displayed in *red*). The percent sequence identity is indicated at the branches. The lid domains are coloured in *green*. All proteins contain variable N-terminal extension, such as the phosphatase domain (*blue*) in case of the soluble epoxide hydrolase, membrane anchors (*grey*) or the N-terminal meander of microsomal epoxide hydrolase (*yellow*)

Table 1 Nomenclature of mammalian α/β hydrolase fold epoxide hydrolases

Gene description present	Recommended	Protein description recommended
EPHX1 (HYL1)	EPHX1	Microsomal epoxide hydrolase, mEH
EPHX2 (HYL2)	EPHX2	Soluble epoxide hydrolase, sEH
ABHD9	EPHX3	Epoxide hydrolase 3, EH3
ABHD7	EPHX4	Epoxide hydrolase 4, EH4
Peg1/MEST	(EPHX5, possibly)	MEST

Peg1/MEST should only be termed EPHX5 when the MEST protein has confirmed epoxide hydrolase activity. We do not recommend involving non-α/β hydrolase fold EHs, e.g. Leukotriene A_4 hydrolase (LTA_4H), or other mammalian EHs like Hepoxilin A_3 hydrolase and Cholesterol epoxide hydrolase (ChEH) in the respective nomenclature, unless sequence information is available

EPHX Epoxide hydrolase xenobiotic, *HYL* hydrolase, *ABHD* α/β hydrolase, *Peg1/MEST* paternally expressed gene 1/mesoderm specific transcript

the histidine), located on top of certain loops (Fig. 3). In the primary sequence the order of the catalytic triad is nucleophile-acid-histidine (Arand et al. 1994). The lid domain presents two tyrosine residues which position the epoxide within the active site. These two tyrosines distinguish epoxide hydrolases from other members of the α/β hydrolase fold family. The latter fold is ideally suited to a detoxifying enzyme that needs to be able to turn over a large number of structurally diverse epoxides (Arand et al. 2003a), because the catalytic nucleophile is positioned at the top of a flexible turn and therefore able to accept structurally diverse epoxides. A negative charge which is formed on the carbonyl oxygen of the catalytic nucleophile (see mechanism below) is stabilised further by a backbone amide of a conserved sequence stretch HGXP (where X is usually aromatic) called oxyanion hole. Apart from these motifs, the overall sequence variation is high which is typical for this type of enzymes (a comparison of the EHs from *A. radiobacter* and *A. niger* shows little sequence similarity but the backbones of their α/β hydrolase fold domains are nearly perfectly superimposable). To date the structures of several α/β hydrolase fold epoxide hydrolases, in particular of murine (Argiriadi et al. 1999) and human (Gomez et al. 2004) sEH, but also of plant (Mowbray et al. 2006), fungal (Zou et al. 2000) and bacterial enzymes (Nardini et al. 1999) have been solved.

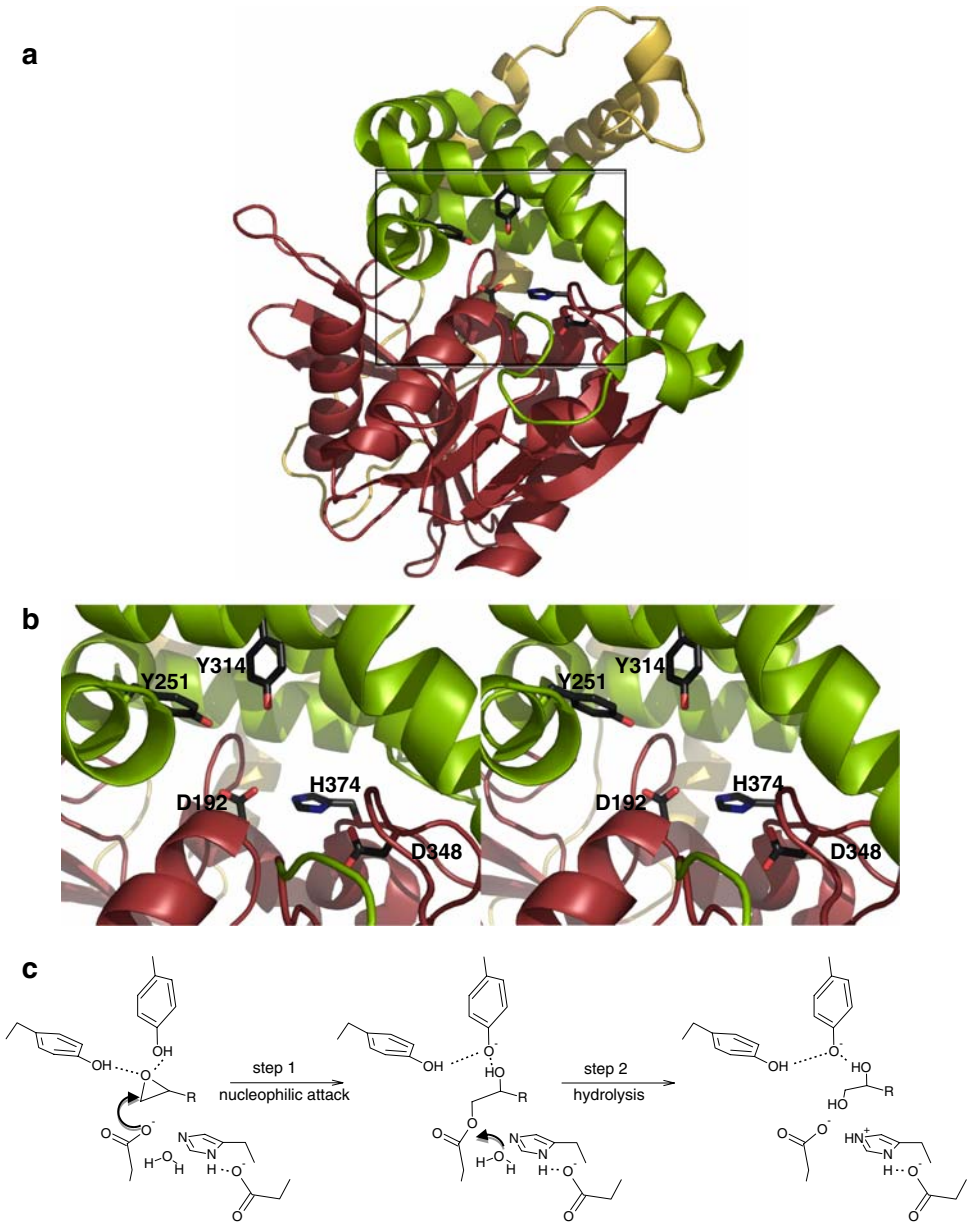

Fig. 3 Three dimensional structure and enzymatic mechanism of α/β hydrolase fold EHs. The structure model represents the *Aspergillus niger* epoxide hydrolase, a close relative of the mammalian mEH, prepared using the program PyMOL (v9.07; DeLano Scientific LLC, San Carlos, CA, USA) based on the coordinates from PDB data file 1QO7. **a** Complete subunit with the α/β hydrolase fold displayed in *red*, the lid domain in *green* and the N-terminal meander in *gold*. **b** Stereo view of the mEH active site, showing the residues relevant for catalysis. **c** Two step catalytic mechanism of epoxide hydrolysis as described in detail in the text

The N-terminal domain varies among mammalian epoxide hydrolases (Arand et al. 2003a) (Fig. 2). While the α/β hydrolase fold in mEH and most likely EH3 and 4 is preceded by a membrane anchor, the N-terminal domain of mammalian sEH acts as a phosphatase and structurally belongs to a distinct class of haloacid dehalogenases (HAD). Structure and function of this additional domain is detailed in the respective sEH chapter below.

Catalytic mechanism of epoxide hydrolysis

Much work has been invested in elucidating the catalytic mechanism of epoxide hydrolases (Arand et al. 1996; Muller et al. 1997; Laughlin et al. 1998; Argiriadi et al. 1999; Armstrong and Cassidy 2000; Zou et al. 2000; Arand et al. 2003c; Elfstrom and Widersten 2006) which is well understood by now. The overall reaction catalyses the addition of water to the oxirane ring leading to a vicinal diol as reaction product, including the formation of an ester intermediate as outlined in Fig. 3c. As described above the active site is composed of a catalytic triad build from the active site residues aspartic acid (catalytic nucleophile), histidine (general base) and aspartic or glutamic acid (charge relay acid). The catalytic triad is supported in catalysis by the two tyrosine residues coming from the lid. The hydroxy groups of these tyrosines hydrogen bond to the epoxide oxygen, once the substrate has entered the active site, and thereby position and activate the epoxide for the catalytic reaction. In the first chemical reaction step the catalytic nucleophile attacks the oxirane ring (with certain stereo- and enantioselectivity depending on the enzyme) at one of the two carbon atoms to form a covalent intermediate (Lacourciere et al. 1993). In a second step the enzyme substrate ester intermediate is then hydrolysed by a water molecule, which is activated by proton abstraction from the histidine of the catalytic triad. The resulting positive charge is shielded by the acidic amino acid residue, either a glutamic (in mammalian EHs) or aspartic acid (in most other EHs) and the oxyanion hole stabilises the intermediate state. The catalytic nucleophile is located on a nucleophilic elbow, and can therefore adapt to the position of the substrate epoxide with some flexibility. This setting represents the ideal active site for broad substrate specificity.

Such a mechanism has some important implications in the detoxification capacity of epoxide hydrolases (Arand et al. 2003c). The rate limiting step of the overall reaction is the hydrolysis of the ester intermediate (Laughlin et al. 1998; Arand et al. 1999a) and release of a diol product. However, in the first reaction step—which represents substrate consumption—the mEH is efficiently able to detoxify a variety of substrates, although with low *Vmax*.[1] The latter fact is accomplished by the fortunate high expression level of mEH in the liver and a generally low concentration of epoxide substrates. Nonetheless, if the formation rate of a reactive epoxide intermediate exceeds the elimination capacity of the mEH, its steady state level is escaping the control of the enzyme. This may represent the mechanistic basis of a practical threshold for chemical carcinogenesis by compounds that are detoxified by mEH (Oesch et al. 2000).

The catalytic addition of water to oxiranes by α/β hydrolase fold epoxide hydrolases as described above is not the only possible mechanism to metabolise epoxides. To make matters complete, three other mechanisms for epoxide hydrolysis have evolved. Mammalian LTA_4 hydrolase (Haeggstrom et al. 2002) as well as the FosX epoxide hydrolases from *Mesorhizobium loti* (Fillgrove et al. 2003; Rigsby et al. 2005) are both metalloenzymes that activate the oxirane by coordination with Zn^{2+} and Mn^{2+}, respectively. While FosX hydrolyses its substrate by direct addition of water, LTA_4 introduces the water three double bonds afar from the epoxide, resulting—as an exception—in a non-vicinal diol. A one step mechanism of hydrolysis is utilised by some bacterial enzymes—LEH from *Rhodococcus erythropolis* and TbEH1 from *Mycobacterium tuberculosis* (Arand et al. 2003b; Johansson et al. 2005)—which form an active site containing the catalytic residues Asp-Arg-Asp (Arand et al. 2003a). Compared to the broad substrate spectrum of mEH, all latter enzymes show rather narrow substrate selectivity.

Mammalian α/β hydrolase fold epoxide hydrolases

Microsomal epoxide hydrolase (EC 3.3.2.9)

Gene and protein structure

The human EPHX1 gene is located on the long arm of chromosome 1 and approximately 20 kb in size, composed of eight introns and nine exons, of which exons 2–9 are coding (Falany et al. 1987). In mammals, several alternative non-coding exons 1 exist, that provide the possibility of

[1] In an enzymatic reaction involving a covalent intermediate the Michaelis constant K_m resolves to $K_m = K_D \times k_2/(k_1 + k_2)$ (where k_1 describes formation of the intermediate, whereas k_2 describes the product formation) and in the case of k_2 being orders of magnitudes smaller than k_1 as mentioned above, k_2 becomes negligible in the denominator, and the ratio K_m/K_D equals the ratio k_2/k_1. K_m is therefore orders of magnitudes smaller then the dissociation constant, which is mimicking a high affinity of the enzyme for its substrate as detailed in Arand et al (2003c) Detoxification strategy of epoxide hydrolase—the basis for a threshold in chemical carcinogenesis. EXCLI J 2:22–30.

recruiting alternative promoters for mEH expression (Gaedigk et al. 1997).

EPHX1 translates into a 455 amino acid polypeptide containing an N-terminal membrane anchor (Friedberg et al. 1994b). A crystal structure of human mEH is not available, however, based on the X-ray structure of a related enzyme from *Aspergillus niger* (which lacks the N-terminal membrane anchor) a structural analysis of mammalian mEH (Arand et al. 1999a; Zou et al. 2000) was performed. The typical α/β hydrolase fold is covered by a lid domain, but unique for mEH compared to other α/β hydrolases is an N-terminal meander that clasps around both core and lid domain (Fig. 3a). Because the Aspergillus EH represents a dimer, it is intriguing to speculate that human mEH may also exist in dimeric form. The mEH active site was analysed by site directed mutagenesis of mEH protein expressed in *E. coli* (Laughlin et al. 1998; Tzeng et al. 1998) and *S. cerevisiae* (Arand et al. 1996), which identified the catalytic triad as Asp226, Glu404 and His431 and the two tyrosine are represented by residues Tyr299 and 374 (Armstrong and Cassidy 2000). Microsomal EHs are the only epoxide hydrolases containing a glutamate as acidic residue. Replacing Glu with Asp leads to an mEH variant so far not found in the animal kingdom with a 30-fold increase in the turnover rate (Arand et al. 1999b). This raises the question why evolution would favour the apparently "inefficient" enzyme variant. One explanation might be that a strong enhancement of the mEH turnover rate could have some undesired effects on endogenous EH substrates (e.g. signalling lipid epoxides) with potential side effects for the organism.

Organ and cellular distribution

Microsomal epoxide hydrolase is highly expressed in the liver and other organs such as the lungs, kidneys, intestine, brain, prostate, heart and testes (Coller et al. 2001). Because mEH seems under a complex transcriptional control due to the utilisation of different promoters, the mEH expression level might vary in dependence on tissue and cell type (Gaedigk et al. 1997). The human mEH expression is inducible by a number of compounds including phenobarbitone and *N*-acetylaminofluorene (Fig. 4c) (Astrom et al. 1987; Hassett et al. 1989), effects mediated by the transcription factors Nrf2 (Kwak et al. 2001), GATA-4 (Zhu et al. 2004) and CAR (Merrell et al. 2008). The protein is attached to the cytosolic site of the endoplasmic reticulum membrane with its N-terminal membrane anchor

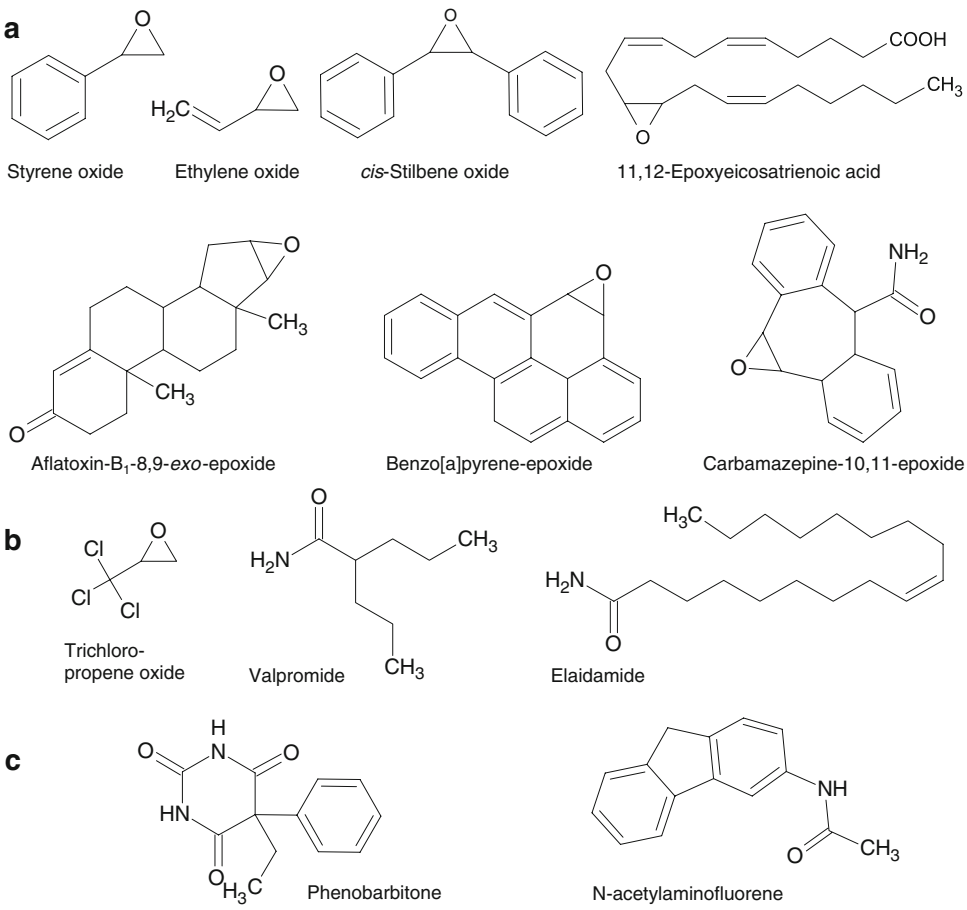

Fig. 4 The substrate spectrum of microsomal epoxide hydrolase. **a** Substrates for mEH. **b** Inhibitors of microsomal epoxide hydrolase. **c** Inducers of microsomal epoxide hydrolase

(Friedberg et al. 1994a; Holler et al. 1997), which correlates to the membrane topology of CYPs. Some reports though claim a localisation of mEH in the plasma membrane, based on the enzymes possible function in bile acid transport (Zou et al. 2000; von Dippe et al. 2003) which is, however, a matter for debate (Honscha et al. 1995).

Physiological functions

The mEH classically plays a major role in xenobiotic metabolism due to its exceptionally broad substrate selectivity and prominent expression in the liver and other metabolising organs, ensuring widespread defence against potential genotoxic epoxides. Due to the special mechanistic features described above, the mEH is involved in the efficient detoxification of many reactive epoxide intermediates (Armstrong 1987; Arand et al. 2003c) including metabolites of polycylic aromatic hydrocarbons (Oesch 1973). Generally good substrates for the mEH are lipophilic substituted epoxides of *cis*-configuration such as *cis*-stilbene oxide. Examples for mEH substrates are epoxides derived from anticonvulsive drugs (carbamazepine-10,11-epoxide) (Bellucci et al. 1987), toxic and procarcinogenic compounds such as styrene-7,8-oxide (Oesch 1974), epoxide derivatives of butadiene (Krause et al. 1997), benzene (Snyder et al. 1993), naphthalene, anthracene and other polycyclic aromatic hydrocarbons (Bentley et al. 1976; van Bladeren et al. 1985; Shimada 2006) (Fig. 4a). The breakdown of such potential genotoxic epoxide to less harmful metabolites is an efficient process in the protection of macromolecules from the electrophilic attack of reactive intermediates.

The efficient detoxification of styrene oxide shows the detoxification capacity of human mEH (Oesch et al. 2000). Styrene-7,8-oxide, which is the major genotoxic metabolite of the industrial chemical styrene formed by CYPs in the human liver (Sumner and Fennell 1994), is efficiently metabolised by mEH to its less toxic metabolite phenyl glycol. However, already a low exogenous exposure to styrene oxide (but not styrene) correlates with protein/DNA adduct biomarkers for styrene oxide exposure (Rappaport et al. 1996). This is best explained by the assumption, that endogenously formed styrene oxide is efficiently hydrolysed immediately after generation without leaving the liver, while styrene oxide of exogenous origin first has to reach this organ via the circulation. This hypothesis is compatible with the results from toxicokinetic models regarding styrene and styrene metabolite exposure in rats, mice and humans (Csanady et al. 2003). Furthermore, lung fibroblasts engineered to express human mEH at the same level as the human liver are well protected against the genotoxic effects of styrene-7,8-oxide after extended exposure, in contrast to the corresponding parental cells that lack human mEH (Herrero et al. 1997).

Under some circumstances the mEH is involved in the toxification of its substrates, with potentially fatal outcome. Such a dual role of mEH is highlighted by its important role in both detoxification and bioactivation of the polycyclic aromatic hydrocarbon benzo[a]pyrene (Shou et al. 1996). While the enzyme can detoxify many CYP derived epoxides, in particular bay region dihydrodiol epoxides of PAHs are no substrates for mEH, which consequently display a highly genotoxic potential (Friedberg et al. 1994a). As displayed in Fig. 5 the enzyme catalyses the regioselective addition of water to the 8-position of (7R,8S)-benzo[a]pyrene-7,8-epoxide. The resulting dihydrodiol is still a good substrate for the oxidation at the 9,10-position to result in the ultimate cancerogen (7R,8S,9S,10R)-benzo[a]pyrene-7,8,9,10-tetrahydro-7,8-diol-9,10-epoxide, which is not a substrate for mEH anymore (Holder et al. 1974). A contentious issue still is as to whether mEH is protective or toxifying in the case of PAHs. Analysis in mEH knock out models may produce valuable answers, but it should be kept in mind that the outcome of results may heavily depend on the chosen experimental model. Reduced carcinogenicity of 7,12-dimethyl benz[a]anthracene (DMBA) in mEH knockout versus control animals was shown in a skin tumourigenicity model (Miyata et al. 1999), and reduced immunosupression of DMBA was reported after systemic application in KO versus control animals (Gao et al. 2007). With another model substrate, such as benzo[a]pyrene, the ratio of formation and elimination of genotoxic metabolites may be different and it is not predictable whether systemic toxicity will be higher or lower in mEH knockout mice compared to control littermates. mEH null mice have further been used to show a protective effect of the enzyme against butadiene (Wickliffe et al. 2007), but genotoxicity and haematotoxicity of benzene are again enhanced in these animals (Recio et al. 2005). Another controversial question is the detoxification capacity of mEH for aflatoxin-B_1-8,9-*exo*-epoxide, the short lived but highly genotoxic metabolite of aflatoxin B_1 produced by *Aspergillus flavus* (a fungus particularly contaminating corn and peanuts). A high incidence of hepatocellular carcinomas is observed in regions with high incidences of hepatitis in combination with high aflatoxin exposure (McGlynn et al. 1995). The turnover rate for aflatoxin-B_1-8,9-*exo*-epoxide with human mEH is rather slow in vitro compared to the spontaneous hydrolysis (Guengerich et al. 1998), but due to the afore mentioned mechanism the protective capacity of mEH may be underestimated. Several studies have connected the susceptibility towards aflatoxin induced hepatocarcinogenesis to polymorphisms in the mEH gene (McGlynn et al. 1995; Wild et al. 2000; McGlynn et al. 2003; Dash et al. 2007). Furthermore, mEH has been reported to provide some protection against the mutagenicity of aflatoxin-B_1-8,9-*exo*-epoxide in recombinant *S. cerevisiae* (Kelly et al. 2002).

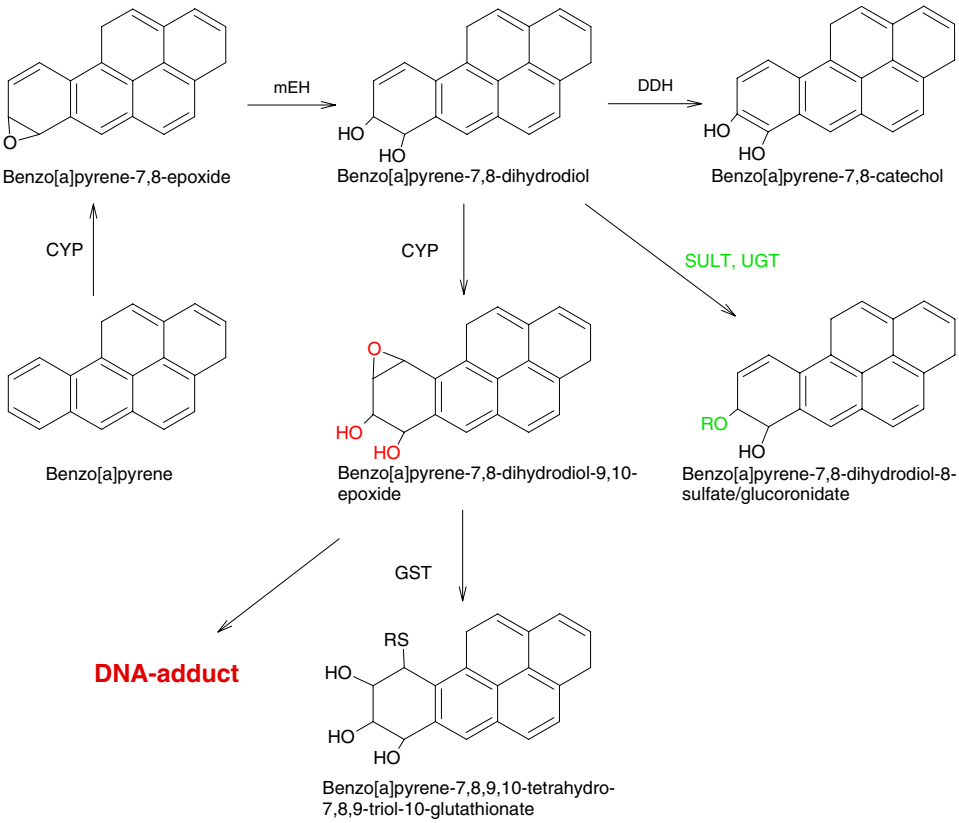

Fig. 5 Metabolism of benzo[a]pyrene. The *representation* shows the metabolic activation of benzo[a]pyrene to benzo[a]pyrene-7,8-dihydrodiol-8,9-epoxide, the ultimate carcinogenic metabolite, which is no substrate for mEH anymore. The other pathways describe metabolic reactions by phase II enzymes leading to detoxification of reactive reaction intermediates

On the other hand, endogenous functions of mEH have long been widely overlooked. Endogenous substrates for mEH are certain steroids like estroxide (Fandrich et al. 1995) or androstene oxide, suggesting a role in development. Latest reports also connect mEH to neurodegenerative disorders such as Alzheimers and Parkinsons disease (Liu et al. 2006, 2008). Despite the fact that the sEH is the main enzyme to metabolise numerous endogenous fatty acid derived epoxides (see below), mEH also accepts fatty acid derived epoxides such as epoxystearic acid with high enantioselectivity (Zeldin et al. 1996), EETs (Oliw et al. 1982) or an epoxide of anandamide (Snider et al. 2007), although generally to a much lesser extent than sEH. Therefore, a role of mEH in signalling cascades cannot be excluded, particularly in the case of a high mEH expression in certain organs or cell types.

A number of mEH inhibitors have been developed to investigate mechanistic aspects of epoxide hydrolysis or an involvement of the enzyme in pathophysiology. 1,1,1,-Trichloropropene-2,3-oxide was found to be an inhibitor of rodent and human mEH (Papadopoulos et al. 1985) and cyclopropyl oxiranes are competitive reversible inhibitors for mEH (Prestwich et al. 1985) (Fig. 4c). Also the anticonvulsant valpromide was identified as a mEH inhibitor and recently other substituted fatty acid amides (elaidamide) were developed as metabolically stable mEH inhibitors of much higher affinity (Morisseau et al. 2001, 2008).

Polymorphisms

Two prominent genetic polymorphisms (Tyr113His and His139Arg) have been identified in the coding region of mEH (Omiecinski et al. 2000), both displaying ethnic differences. The protein variants resulting from the different haplotypes have been reported to display different half lives (Hassett et al. 1994, 1997), so that these genetic variants may have impact on mEH activity in vivo. mEH polymorphisms may lead to differences in bioactivation of procarcinogens, resulting in altered susceptibilities to cancers of various tissues (Tranah et al. 2005; Lin et al. 2007; Mittal and Srivastava 2007). Studies associating mEH polymorphism with lung cancer have obtained apparently contradictory results (Habalova et al. 2004). However, two meta analysis associated the His113/His139 haplotype (predicted slow activity) with a significantly decreased risk of lung cancer (Lee et al. 2002; Kiyohara et al. 2006). Other studies associate the His113/His139 variant with a protective effect regarding chronic obstructive pulmonary disease (Brogger et al. 2006), as well as a decreased risk of pregnancy induced hypertension (preeclampsia) (Zusterzeel et al. 2001), but on the other hand an increased susceptibility to development of liver cancer after exposure to aflatoxin-B1 (Hengstler et al. 1998; McGlynn et al. 2003) was demonstrated. The His113/His139 variant further has been reported to be more frequent in COPD and lung emphysema patients

as compared to healthy controls (Smith and Harrison 1997). Moreover, several other polymorphisms have been identified in the 5′-flanking region of the mEH promoter region (Raaka et al. 1998).

Soluble epoxide hydrolase (EC 3.3.2.10, EC 3.1.3.76)

Gene and protein structure

The human EPHX2 gene is located on chromosome 8, approximately 54 kb in size, and composed of 19 exons and 18 introns (Sandberg and Meijer 1996). Two alternative ovary specific transcripts EPHX2B and C have recently been described, that both substitute the first two exons of the known sEH sequence (Hennebold et al. 2005; Shkolnik et al. 2007). This results in a sEH protein which lacks essential catalytic components of its N-terminal phosphatase domain (see below), while leaving the epoxide hydrolase domain intact. These alternative transcripts are particularly interesting as the sEH knockout line (Sinal et al. 2000) was constructed by disruption of the first intron, whereas in a second established knock out line (Luria et al. 2007) the exact disruption site is not published. Thus, the knock out may be incomplete in both cases, with respect to the epoxide hydrolase activity of the enzyme.

Mammalian sEH has a primary structure of 554 amino acids (Beetham et al. 1993; Grant et al. 1993; Knehr et al. 1993). One sEH monomer is constituted of two separate catalytic domains—a phosphatase in the N-terminus and the epoxide hydrolase in the C-terminus—separated by a proline rich linker (Fig. 6a). Mammalian sEH is a homodimeric enzyme showing a domain swapped architecture, where the N-terminal domain of one subunit interacts with the C-terminal domain of the other. Both domains are classified within large and separate superfamilies of hydrolases based on structural similarities. The structures of murine (Argiriadi et al. 1999) and human (Gomez et al. 2004) sEH have been solved giving interesting insights into the mechanism of the enzyme. The C-terminal domain (325 amino acids) of each subunit functions as a α/β hydrolase fold epoxide hydrolase as described earlier. The catalytic triad of rodent sEH is composed of the active site residues Asp333, Asp495 and His523 (Pinot et al. 1995; Arand et al. 1996) and the two tyrosines Tyr382 and Tyr465 (Argiriadi et al. 1999).

The smaller N-terminal domain instead displays a recently discovered phosphatase activity (Cronin et al. 2003; Newman et al. 2003). This phosphatase domain belongs to a distinct family of HADs, comprising a number of phosphatases, dehalogenases and other hydrolases (Koonin and Tatusov 1994; Beetham et al. 1995). Albeit an overall low sequence similarity, all enzymes of the HAD superfamily possess a structurally conserved rossmannoid fold (core domain), containing four loops that form the catalytic scaffold (Fig. 6a). Loop 1 contains the catalytic Asp nucleophile followed by a second aspartic acid (Wang et al. 2002; Allen and Dunaway-Mariano 2004). Loops 2 and 3 orientate the phosphate substrate with residues Ser/Thr and Lys/Arg, whereas loop 4 contains the Mg^{2+} binding pocket formed by two aspartic acids (Baker et al. 1998; Morais et al. 2000; Wang et al. 2001). Additionally, the HAD enzymes are divided into three subfamilies I–III due to the presence and location of an additional cap domain. Class I HADs such as the sEH phosphatase contain an insertion between loop 1 and 2 of the core domain (Lahiri et al. 2004, 2006). This cap domain is implicated in solvent protection of the active site as well as substrate selectivity (Selengut 2001). Based on structural similarities with other members of the HAD family and site directed mutagenesis we identified the composition of the active site as Asp9, Asp11, Thr123, Asn124, Lys160, Asp184, Asp185 and Asn189. We recently investigated the catalytic mechanism of dephosphorylation, using kinetic evaluation of active site mutants and LC–MS/MS analysis of the phosphorylated enzyme intermediate. In the first step in the dephosphorylation reaction, the phosphate residue is transferred from the substrate to Asp9 which acts as the catalytic nucleophile (Fig. 6b, c). In a second step the phosphoenzyme intermediate is hydrolysed via nucleophilic attack of an activated water molecule (Cronin et al. 2008).

Cellular distribution

The sEH is expressed in almost every organ such as liver, lungs, kidneys, heart, brain and ovary (Enayetallah et al. 2004; Sura et al. 2008). The enzyme is mainly localised in the cell cytosol, but in some cell types sEH shows a dual localisation, both cytosolic and peroxisomal. This is due to an imperfect C-terminal peroxisomal targeting sequence (PTS-1) (Arand et al. 1991; Mullen et al. 1999) and in dependence of protein expression level and quaternary structure (Enayetallah et al. 2006a; Luo et al. 2008).

In rodents, sEH expression is inducible by peroxisome proliferators (PPs, a number of structurally diverse compounds including hypolipidemic drugs such as fibrates and phthalates) (Fig. 7c), an effect mediated by the transcription factor peroxisome proliferator activated receptor (PPAR) (Oesch et al. 1986; Lundgren et al. 1987; Johansson et al. 1995), which suggests a possible role of sEH in PP induced liver cancerogenesis. A PPAR mediated induction of sEH in humans has not yet been detected. Recently, an AP1 mediated regulation of sEH by Angiotensin-II has been shown (Ai et al. 2007), and further Monti et al. (2008) identified an sEH genetic variant associated with heart failure in rats, which is characterised by the existence of a new AP1-binding site in the promoter region.

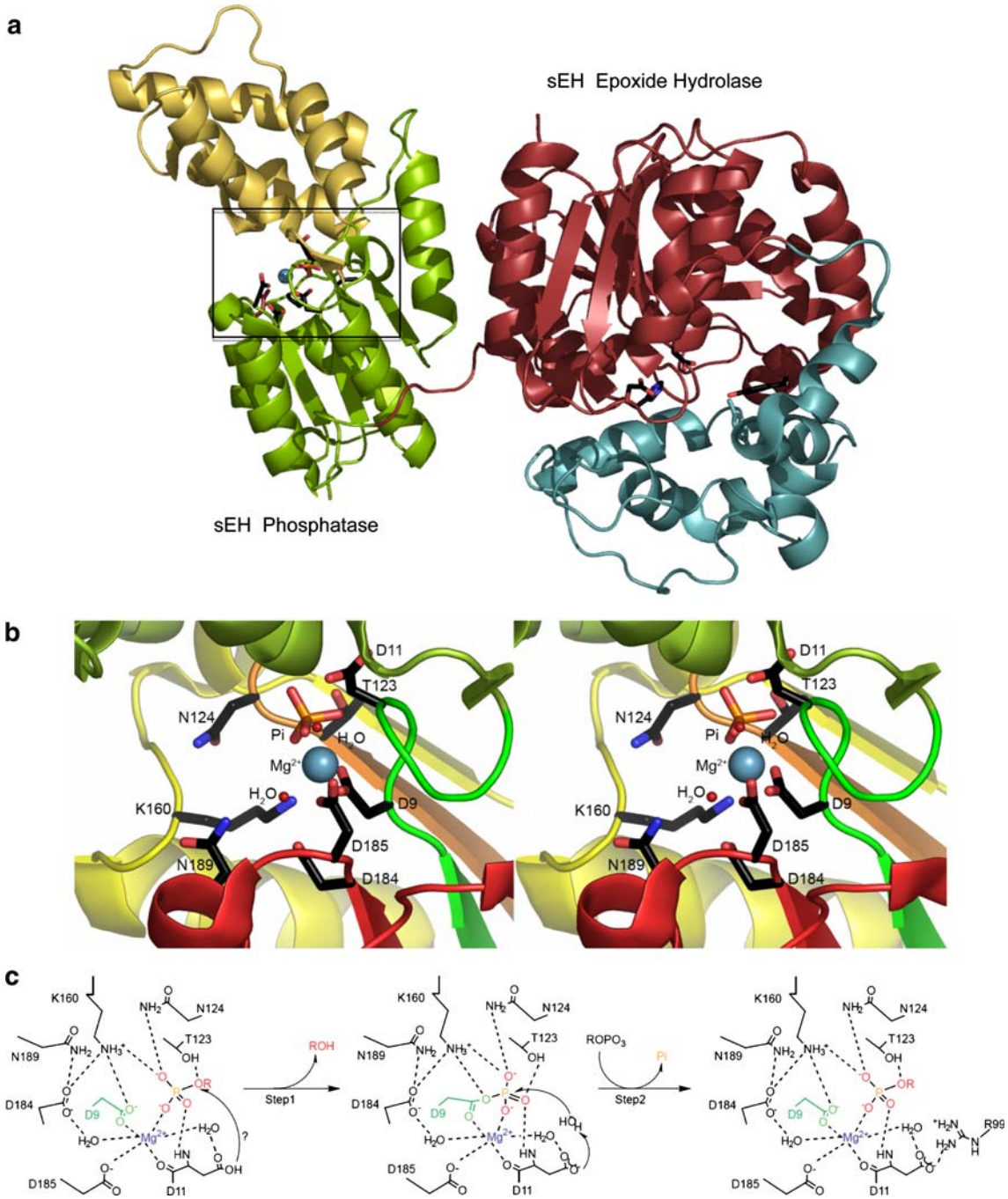

Fig. 6 Three dimensional structure of soluble epoxide hydrolase and catalytic mechanism of the sEH phosphatase. **a** Structure model of one subunit of the homodimeric soluble epoxide hydrolase, consistent of an N-terminal phosphatase (Core domain represented in *green* and lid domain in *gold*) and the C-terminal epoxide hydrolase domain (α/β hydrolase fold displayed in *red* and lid domain in *blue*). **b** Stereo view of the sEH phosphatase active site, with the catalytic amino acids highlighted. The structural model was prepared using the program PyMOL (v9.07; DeLano Scientific LLC, San Carlos, CA, USA) based on the coordinates from the PDB data file 1CQZ. **c** Two step mechanism of dephosphorylation as described in detail in the text

Physiological functions

Most established sEH functions are currently assigned to the epoxide hydrolase activity. In xenobiotic metabolism the sEH complements the mEH spectrum of substrates for an efficient turnover, in that it hydrolyses *trans*-substituted slender epoxides and shows complementary substrate specificity to mEH (Morisseau and Hammock 2005), including potentially harmful epoxides (Arand et al. 2003a). Typical substrates are *trans*-stilbene oxide, and in particular fatty

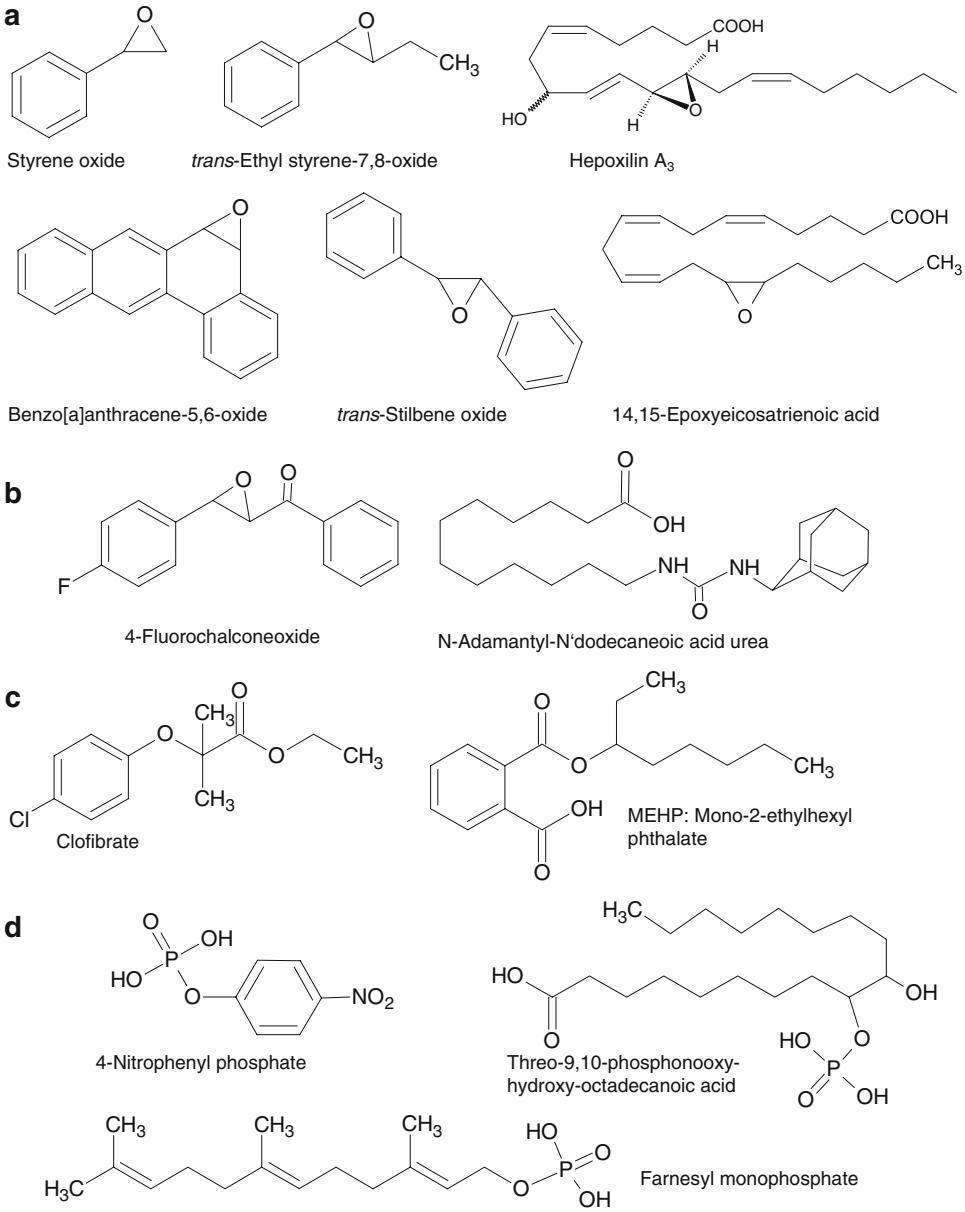

Fig. 7 The substrate spectrum of soluble epoxide hydrolase. **a** Substrates of soluble epoxide hydrolase. **b** Inhibitors of soluble epoxide hydrolase. **c** Inducers of soluble epoxide hydrolase. **d** Substrates of the sEH phosphatase

acid derived substrates (Fig. 7a). Fatty acids such as *cis*-9,10-epoxystearic acid have been identified as excellent substrates for mammalian sEH (Zeldin et al. 1993; Summerer et al. 2002), but in contrast to soybean sEH or mammalian mEH with little enantioselectivity or enantioconvergence (Summerer et al. 2002).

In contrast to a classical role in detoxification processes, the major physiological role of sEH certainly is the metabolism of fatty acid derived epoxides to the corresponding diols. The organism utilises a large number of endogenous epoxides—mainly derived from arachidonic acid (EETs) and linoleic acid (leukotoxin)—as important signalling molecules and physiological regulators. One of the first reports of physiological relevance was the sEH catalysed turnover of leukotoxin to the actual toxic metabolite leukotoxin diol, causing multiple organ failure and adult respiratory distress syndrome after severe body burns (Moghaddam et al. 1996). Human sEH is the primary enzyme that metabolises such endogenous epoxides (Moghaddam et al. 1996; Morisseau and Hammock 2005; Newman et al. 2005), although other mammalian epoxide hydrolases may play a physiological role under certain conditions.

Due to its important role in the lipid metabolism of EETs, the sEH has recently evolved as a target for the treatment of hypertension (Fang et al. 2001; Imig 2005; Newman et al. 2005), inflammatory diseases (Liu et al. 2005; Schmelzer et al. 2005; Inceoglu et al. 2006), and a novel treatment of pain, diabetes and stroke (Ohtoshi et al. 2005; Schmelzer et al. 2006; Spector and Norris 2007; Zhang

et al. 2007). The formation of *cis*-EETs is catalysed by various CYPs and sEH hydrolyses all EET regioisomers with the order of preference being 14,15-EET > 11,12-EET > 8,9-EET > 5,6-EET. The breakdown of EETs by sEH is in general believed to be a deactivation process. EETs were identified as endothelium derived hyperpolarisation factor, causing prostacyclin and NO independant vasodilation in vascular beds (Fisslthaler et al. 1999), which created massive interest in these lipid derived epoxides. EETs (released from the endothelium) act on large conductance calcium activated K^+ channels (BKCa) on vascular smooth muscle cells leading to hyperpolarisation and vasodilation (Hu and Kim 1993; Li and Campbell 1997) by a mechanism including $G_{\alpha s}$ activation (Li and Campbell 1997). To complicate matters, the different EET regioisomers may have opposing effects dependant on the cell type and experimental set up, and EET effects might be stereoselective (Imig et al. 1996; Falck et al. 2003; Pomposiello et al. 2003; Newman et al. 2005). Despite contradictory reports on the influence of sEH gene disruption on basal blood pressure (possibly due to compensation by the vasoconstrictor 20-HETE) in sEH null mice (Sinal et al. 2000; Luria et al. 2007), several experimental hypertensive models have indeed shown a role of EETs in blood pressure regulation and end organ protection, using specific sEH inhibitors (Imig et al. 2002; Zhao et al. 2004; Fang 2006; Li et al. 2008; Zhang et al. 2008a). Very recently the sEH has been shown to be a susceptibility factor for heart failure in a rat model (Monti et al. 2008). Evidence is accumulating that EETs also display anti-inflammatory properties, by a mechanism based on disruption of the proinflammatory Nf_Kb signalling pathway. sEH inhibitors attenuated tobacco smoke induced lung inflammation in rats (Smith et al. 2005), and sEH KO mice also showed a survival advantage following acute systemic inflammation induced by lipopolysaccharides, effects that might at least in part be mediated by PPARγ (Liu et al. 2005; Inceoglu et al. 2006; Schmelzer et al. 2006). The latter studies also pointed to an antinociceptive effect of EETs, as administration of sEH inhibitors decrease LPS-induced thermal hyperalgesia and mechanical allodynia in a systemic model of inflammatory pain (Inceoglu et al. 2006), an effect enhanced by coadministration of sEH inhibitors and common non-steroidal anti-inflammatory drugs (Schmelzer et al. 2006). Very recently 14,15-EET was shown to mediate its antinociceptive effect via the μ- and δ-opioid receptor pathway (Terashvili et al. 2008). Because EETs play important roles in the brain—including regulation of cerebral blood flow and protection from ischemic brain injury—sEH has emerged as a potential pharmacological target for the treatment of stroke (Zhang et al. 2007, 2008b; Gschwendtner et al. 2008). Finally, several studies point to an effect of EETs on cell proliferation (Potente et al. 2003), migration (Sun et al. 2002) and angiogenesis (Michaelis et al. 2005), actions which seem mediated through several signalling pathways including p38 MAPK, PI_3K Akt or PKA and in dependence of the species, the type of endothelium and/or the EET regioisomer (Spector and Norris 2007).

Due to the various described endogenous functions of EETs, inhibitors for sEH (sEHI) have been developed over the past decade, aiming in particular at new therapeutics for the treatment of hypertension. sEHIs comprise of several chemical classes such as chalcone oxide derivatives (Morisseau et al. 1998), trans-3-phenylglycidols (Dietze et al. 1991, 1993), as well as urea and carbamate-based inhibitors (Morisseau et al. 2002, 2006; Kim et al. 2004, 2007) (Fig. 7c).

In contrast, the physiological role of the recently discovered phosphatase activity of sEH is much less clear, but it further highlights the role of this enzyme in regulatory processes. The sEH phosphatase so far accepts the generic substrate 4-NPP, some lipid phosphates (Newman et al. 2003; Tran et al. 2005), as well as isoprenoid phosphates (Enayetallah et al. 2006b; Enayetallah and Grant 2006). Of these *threo*-9,10-phosphonooxy-hydroxy-octadecanoic acid as well as the monophosphates of farnesol and geraniol are among the best substrates (Fig. 7d). Because isoprenoid phosphates are intermediate metabolites at the branching point of cholesterol biosynthesis as well as precursors for protein prenylation, the sEH phosphatase is possibly connected to cholesterol biosynthesis, protein prenylation and metabolism of certain lipid phosphates. Due to the dual localisation of sEH in cell cytosol and peroxisomes, the physiological target of the sEH phosphatase activity might be organelle specific. Recently two ovary specific alternative transcripts—EPHX2B and C—of the sEH have been identified (Hennebold et al. 2005; Shkolnik et al. 2007) both of which are shortened at the N-terminus leading to an inactive phosphatase domain. EPHX2C is found in the ovary at the highest level of expression occurring during the luteal phase of a stimulated oestrous cycle, suggesting a role of the sEH phosphatase in hormonal regulation.

Efforts are being made to develop specific sEH phosphatase inhibitors (Tran et al. 2005; Enayetallah et al. 2006a), but due to the large collection of endogenous phosphatases this seems to be a challenging task.

Polymorphisms

A number of non-synonymous nucleotide polymorphisms (Lys55Arg, Arg103Cys, Cys154Tyr, Arg287Gln, Val422Ala and Glu470Gly, insertion Arg402ArgArg) have been identified for human sEH (Sandberg et al. 2000; Saito et al. 2001; Przybyla-Zawislak et al. 2003) that affect the protein coding sequence as well as enzymatic activity. Of these, sEH variant Lys55Arg, which has increased epoxide hydrolase

activity, is associated with coronary artery disease, especially in Caucasian, but not African American cigarette smokers (Lee et al. 2006). The most significant polymorphism however is represented by sEH variant Arg287Gln which seems to be associated with a number of diseases. The Arg287Gln mutation results in sEH protein with reduced stability and reduced epoxide hydrolase activity (Przybyla-Zawislak et al. 2003), as well as decreased ability of homodimer formation (Srivastava et al. 2004) and therefore facilitated peroxisomal import (Luo et al. 2008). The reported results on phosphatase activity are inconsistent, increased phosphatase activity (Enayetallah and Grant 2006) as well as decreased phosphatase activity of the Arg287Gln mutant as compared to the WT enzymes using isoprenoid phosphates and/or generic substrate (Srivastava et al. 2004) are described. The allelic frequencies for the Arg287Gln allele are 5% for Europeans, 20% for Asians and 8% for Africans. The Arg287Gln allele was associated with increased risks for coronary artery calcification in African Americans, but not caucasians (Fornage et al. 2004; Burdon et al. 2008), insulin resistance in diabetes type II patients (Ohtoshi et al. 2005), ischemic stroke (Koerner et al. 2007), as well as hypercholesterolemia (Sato et al. 2004) in carriers of a LDL-receptor mutation.

EH 3 and 4

The genes EPHX3 and 4 are closely related, the corresponding gene products EH3 and EH4 are polypeptides of 360 and 362 amino acids in length, respectively. EH 3 and 4 show a sequence identity of 45%, with higher sequence similarity to sEH than mEH (Fig. 2). Both proteins contain a predicted N-terminal membrane anchor. No report of an endogenous substrate for EH3 and 4 is available yet, but at least EH3 represents a functional epoxide hydrolase. Substrate spectrum as well as expression pattern of both enzymes are currently being assessed in our group (manuscript in preparation).

The first hints to cellular distribution of EH3 and 4 stem from EST and microarray database analysis, according to which expression of EH 3 is generally low but found in lung, tongue and skin. A recent study of the human epidermal transcriptome demonstrated a high expression of EH3 in human granular keratinocytes (Toulza et al. 2007), and a human/mouse conserved coexpression analysis to predict human disease genes suggests EPHX3 as candidate gene for ichthyosis (Ala et al. 2008). Moreover, EPHX3 gains some attention as methylation marker for cancer prognosis. In many cancers tumour suppressor genes are inactivated by methylation silencing of their promoter regions. CpG islands in the promoter region of EPHX3 are methylated in melanoma cell lines compared to cultured human epidermal melanocytes (Furuta et al. 2006), as well as in primary gastric cancers and multiple gastric cancer cell lines (Yamashita et al. 2006). EPHX3 was further identified as candidate marker for prostate cancer prognosis by showing increased methylation levels in patients with early PSA recurrence compared to non-recurrent patients (Cottrell et al. 2007). These results indicate a transcriptional silencing of the EPHX3 gene in some cancer events.

In contrast, an expression of EH4 is reported mainly in the brain and eyes. Studies in our group (manuscript in preparation) and expression analysis of rat brain (Stansberg et al. 2007) confirm these findings.

Taken together, the expression pattern suggests a role for both enzymes in the regulation of endogenous functions rather than a role in xenobiotic metabolism. Future investigation will show the exact physiological function of both enzymes.

MEST

The paternally expressed gene 1/mesoderm specific transcript (peg1/MEST) is an imprinted gene that is widely expressed in mammalian tissue (Kobayashi et al. 1997). As an imprinted gene, peg1 shows a promoter methylation pattern that reflects a strict parent-of-origin-specific differential methylation (Lefebvre et al. 1997). Expression of peg1/MEST stays imprinted in adult mice and humans and is high in the nervous system, but an occasional loss of imprinting occurs in cases of invasive breast cancer (Pedersen et al. 1999) and lung adenocarcinoma (Nakanishi et al. 2004). The 335 amino acid polypeptide has a predicted N-terminal membrane anchor. An epoxide hydrolase activity of peg1/MEST still needs to be confirmed, however the protein shows significant sequence similarity to α/β fold epoxide hydrolases (Kaneko-Ishino et al. 1995). MEST contains one conserved, probably epoxide-coordinating tyrosine in the primary sequence and two related epoxide hydrolases from *M. tuberculosis* do indeed show weak epoxide hydrolase activity (personal observations).

A physiological function of MEST has not yet been established, but the fact that MEST is an imprinted gene suggests an important role in development. Targeted disruption of peg1/MEST leads to growth retardation and behavioural effects (Lefebvre et al. 1997). Very recently MEST has been associated with fat mass expansion in response to a high fat diet, due to an up to 50-fold upregulation of mRNA and protein levels in adipose tissue (Nikonova et al. 2008), which further suggest a role of MEST in lipid metabolism.

Other mammalian epoxide hydrolases

Hepoxilin epoxide hydrolase (EC 3.3.2.7)

Hepoxilin epoxide hydrolase was partly purified from rat liver cytosol (Pace-Asciak and Lee 1989) and discriminated

from sEH due to its size of 53 kDa and substrate selectivity for hepoxilin A_3, while showing only marginal activity towards leukotriene or styrene oxide. Because Hepoxilin EH is not cloned to date the question remains whether this enzyme belongs to the family of α/β hydrolase fold EHs. A function in xenobiotic metabolism is—despite an expression in the liver—unlikely, due to the rather specific substrate spectrum.

Hepoxilins (hydroxyepoxy eicosanoids) are widely distributed in mammals (Newman et al. 2005; Nigam et al. 2007). The *trans*-epoxides hepoxilin A_3 and B_3 are derived from the action of 12-lipoxygenase on arachidonic acid and may be degraded by the action of an EH (to the corresponding trihydroxy metabolite trioxilin) or conjugation with glutathione (Laneuville et al. 1991). Hepoxilins formed in rat pancreatic islets enhance the glucose dependant secretion of insulin (Pace-Asciak and Martin 1984). Hepoxilins facilitate calcium transport (Derewlany et al. 1984), and they are further formed in the brain with synaptic neuromodulatory actions on hippocampal neurons by hyperpolarisation and enhancement of the inhibitory postsynaptic potential (Pace-Asciak et al. 1990, 1995). Hepoxilin signalling is most likely receptor mediated, an assumption based on the identification of a hepoxilin binding protein in human neutrophils (Reynaud et al. 1999). Hepoxilin A_3 seems to be a key regulator of neutrophil migration in response to an inflammation response (Mrsny et al. 2004). Due to the occurrence of increased hepoxilins and trioxilin levels in psoriatic lesions the lipids are thought to act proinflammatory in the skin (Anton et al. 1998). Moreover, mutations in the hepoxilin generating epidermal lipoxygenase pathway are associated with a congenital form of ichthyosis. This suggests an involvement of these lipid mediators in epidermal differentiation and skin barrier function (Brash et al. 2007; Epp et al. 2007; Yu et al. 2007).

Nonetheless, hepoxilin A_3 and B_3 are excellent substrates for mammalian sEH (manuscript in preparation). The enzyme is further expressed in hepoxilin generating tissue, which strongly suggests a physiological function of sEH in hepoxilin metabolism. The regulation of these signalling molecules might occur by both enzymes depending on cell type/tissue or regulatory state.

Cholesterol epoxide hydrolase (EC 3.3.2.11)

The existence of an epoxide hydrolase specific for cholesterol-5α,6α-epoxide was already described 1974 (Aringer and Eneroth 1974; Chan and Black 1976; Oesch et al. 1984). ChEH is widely distributed in mammals (Astrom et al. 1986), but little is known about the enzyme and no gene sequence is available. An induction of ChEH has been reported in rodents exposed to the PPARα agonist clofibrate (Finley and Hammock 1988), like as for sEH. Although also present in microsomal cell fractions, ChEH activity is distinct from mEH (Oesch et al. 1984). Moreover, several lines of evidence suggest that ChEH does not represent a α,β-hydrolase fold EH. Size exclusion analysis of the native enzyme partly purified from mouse liver suggested ChEH to be smaller than a typical α,β hydrolase fold epoxide hydrolase (Watabe et al. 1986). In contrast to mEH and sEH the enzyme could not be labelled covalently with ^{14}C-cholesterol-5α,6α-epoxide. These results led to the speculation that ChEH might hydrolyse its substrate via a different mechanism and represents the first mammalian EH member similar to the recently described bacterial LEH (EC. 3.3.2.8) from *Rhodococcus erythropolis* (Barbirato et al. 1998). The LEH hydrolyses its substrate in a single step mechanism and further shares structural similarity to an epoxide hydrolase from *Mycobacterium tuberculosum* (TbEH1). Strikingly, this TbEH1 is the only other enzyme able to facilitate such a bulky substrate like cholesterol-5,6-epoxide (Arand et al. 2003a, b; Johansson et al. 2005).

An oxidation of cholesterol proceeds as part of the lipid peroxidation process in membranes (Astrom et al. 1986), and ChEH functions in conversion of 5α,6α-epoxicholestane-3β-ol and 5β,6β-epoxicholestane-3β-ol to cholestane-3β,5α,6β-triol (Watabe et al. 1981). Some toxicological interest arose from cholesterol epoxide which was reported to act as a weak direct acting mutagen (Sevanian and Peterson 1984, 1986). Cholesterol epoxide was further the suspected dermatocarcinogen in hairless mice irradiated by UV. The mutagenicity of cholesterol epoxide in V79 Chinese hamster lung fibroblasts (Black and Douglas 1972; Reddy and Wynder 1977; Sevanian and Peterson 1984) suggests that the ChEH might play a role in protecting cells from these steroid toxicants, however, the cholesterol epoxides are quiet stable and the corresponding cholestantriols are themselves cytotoxic (Wilson et al. 1997; Vejux et al. 2007). Further, cholesterol epoxides are implicated in apoptosis (Ryan et al. 2004), as well as vascular function and coronary artery disease (Rimner et al. 2005) because they are found upregulated in arteriosclerotic lesions. Only a complete characterisation of ChEH can help to fully understand the physiological role of this enzyme.

Leukotriene A_4 hydrolase (EC 3.3.2.6)

As an atypical epoxide hydrolase Leukotriene A_4 hydrolase will only be briefly addressed, and we refer to some recent excellent reviews (Chen et al. 2004; Haeggstrom 2004; Newman et al. 2005; Haeggstrom et al. 2007). The enzyme has been cloned (Funk et al. 1987), purified from rat and human erythrocytes and neutrophiles (Radmark et al. 1984; Evans et al. 1985; McGee and Fitzpatrick 1985), and the crystal structure has been solved (Tsuge et al. 1994; Thunnissen et al. 2001). LTA_4H is not related to mEH, sEH or

other α/β-hydrolases, instead the bifunctional metalloprotein LTA_4H possesses an epoxide hydrolase as well as a aminopeptidase activity (Haeggstrom et al. 1990; Minami et al. 1990).

The aminopeptidase activity is of presently unknown biological function, although limited peptide substrates have been described (Griffin et al. 1992). LTA_4H hydrolyses leukotriene A_4 which is generated via the 5-lipoxygenase pathway from arachidonic acid—not like sEH to the vicinal diol—but to LTB_4, a non-vicinal 5,12-diol (McGee and Fitzpatrick 1985). LTB_4 is a potent chemotactic agent for neutrophiles, eosinophiles, monocytes and T-Cells, which all play key roles in immune response. Therefore LTA_4H is strongly linked to the pathophysiology of arteriosclerosis, myocardial infarction and stroke (Haeggstrom 2004). Because LTA_4H generally functions as a proinflammatory enzyme, LTA_4H has as of late attracted attention as a target for cancer prevention. LTA_4H inhibitors prevented DMBA induced oral carcinogenesis in hamsters (Sun et al. 2006) and inhibited proliferation as well as induced apoptosis in pancreatic adenocarcinomas (Zhou et al. 2007).

Outlook

Albeit the important role of epoxide hydrolases—mEH in particular—in xenobiotic metabolisms it is now evident that epoxide hydrolases have vital roles in endogenous regulatory processes. This seems also the case for the newly identified potential epoxide hydrolases. Only a throughout characterisation of these enzymes including an evaluation of the substrate spectrum and identification of cellular EH substrate receptors will clear their physiological function. The potential of mammalian epoxide hydrolases as drug targets is highlighted by the growing interest in the development of effective epoxide hydrolase inhibitors.

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